PRODUCTION , DETOXIFICATION AND INACTIVATION OF AFLATOXINS DURING CORNSEED OIL PROCESSING

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PREFACE

Aflatoxins are the most important toxic mycotoxins known to man . They were highly toxic secondary mold metabolites which have identified as potent hepatocarcinogens. They have been detected in foodstuffs from many areas of the world. Aflatoxins are mainly produced by many strains of Aspergillus flavus group from which the name aflatoxins was implied.

Aflatoxins were naturally produced in foodstuffs such as peanuts, cotton-sseds, various tree nuts, wheat grains, soybean seeds, barely grains, cassava, cowpeas seeds, millet grains, peas seeds, rice grains, sesame seeds, sorghum grains and sweet potatoes. Contamination of corn seed and other foodstuffs with aflatoxins is a serious economic and health hazard. Attempts to utilize grain containing relatively low levels of aflatoxins often resulted in costs to live stock producers as a results of decreased feed efficiencies, impaired immunity, and other symptoms of acute aflatoxins.

Potential problems relating to human safety occur when contaminated seeds is used. Contamination of cornseed meal with aflatoxins is considered dangerous to milk consumers, because aflatoxins (M) is readily transmitted to milk of dairy cattle fed contaminated.

Various acids, oxidising agents, aldhydes and nitrogen compounds have been tested for the ability to chemically inactive aflatoxins present in the contaminated samples. For large scale detoxification, the chemicals with greatest practical potential apears to be ammonia gas and ammonium hydroxide. Ammonia inactivation of aflatoxins in oilseed meals, particularly cottonseed meal has been reported, by several investigators.

The carcinogenic properties of aflatoxins have reinforced the concept that naturally occuring mycotoxins may be involved in the etiology of human cancer on a broad basis.

Thus, the present investigation is conducted mainly to study the contamination of corn-seed oil during its processing by aflatoxins. This study is also focused on the detoxification of aflatoxins contamination of crude oil by removal or inactivation.

PART I

A. HISTORICAL INTRODUCTION

B. MATERIALS AND METHODS

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OCCURRENCE OF AFLATOXINS :

Since the review of Hesseltine et al., (1966) which cited litrature through 1964, aflatoxin has been found to be naturally occurred in commodities other than peanuts and cottonseed cake (Loosmore et al., 1964). It is highly probable that cases of moldy corn poisoning in Georgia in the early 1950's were the result of aflatoxins (Burnside et al., 1957 & Forgacs, 1962). Moldy corn was associated with the death of swine on several occasions during (1964-1968) in Alabama. Samples of this corn contained aflatoxin B_1 with concentrations ranging from 80 to 8733 ppb. Since corn is widely grown throughout the United States and on the farm storage for feeding and for holding for market is a common practics, it is possible that the hazard of aflatoxins in corn may exceed that in peanuts, rice, and cottonseed. They have demonstrated the presence of aflatoxins in coastal bermudagrass hay, sovbeen meal, oats, and cottonseed meal by TLC.

According to Borker et al., (1966) aflatoxins has been found as natural contaminats in many agricultural commodities including cassava, corn, cottonseed meal, peanuts, peanut meal, peas, rice, soybeans, and wheat. In food samples collected from various parts of the world, particularly from Africa and Asia, aflatoxins were detected at biologically significant levels in a wide spectrum of commdities including barely, cassava, corn, cottonseed, cowpeas, millet, peanuts, peas, rice, sesame, sorghum, soybeans, sweet potatoes, and wheat (Wogan, 1968). Aflatoxins has been found to occur naturally on Brazil nuts and dry spaghetti (Walbeek *et al.*, 1968). Aflatoxins has also been found in the poorer grades of samples of commercial oat, wheat, corn, soybean, and grain sorghum seed (Shotwell *et al.*, 1969a). It is likely that aflatoxins will continue to be found in food and feed-stuffs, wherever warm and moist weather conditions , faulty or inadequate storage facilites, and human error or ignorance combine to produce circumstances favorable for fungal growth.

The occurrence of aflatoxins other than B_1, B_2, G_1 and G_2 have been found in extracts from milk and urine of animals and from cultures of *A. flavus* grown on natural and synthetic substrates. Allcroft and Carnaghan (1963) reported that extracts of milk from cows fed on aflatoxins-containing groundnut meal induced liver lesions identical to those caused by aflatoxins in ducklings. TLC examination showed that there was no aflatoxin B_1 present. This "milk toxin" was shown to be identical to a blue-violet fluorescent component also present in toxic groundnut meal (de Iongh *et al.*, 1964), and it was found that the lactating rats converted pure aflatoxin B_1 into the milk toxin. Butler and Clifford (1965) have found the milk toxin in the liver of rats fed aflatoxin B_1. Allcroft *et al.*, (1966) designated the milk toxin as aflatoxin M after finding it in the liver, kidney, and urine of sheeps dosed with a mixture aflatoxins B_1, B_2, G_1 and G_2. Holzapfel *et* al., (1966) isolated aflatoxin M from sheeps urine and separated two components designated as M_1 and M_2. They determined their structures and concluded that M_1 was hydroxy aflatoxin B_1 and that M_2 was dihydroxy aflatoxin B_1. Dutton and Heathcote (1966) reported that two metabolities isolates from cultures of A. flavus were hydroxy derivatives of aflatoxins B_2 and G_2, and designated them as Ba and Ga. Later, they eluciadated the structure and biochemical properties of aflatoxins B_2a and G_2a, and found them to be much less toxic to ducklings than the other aflatoxins (Dutton & Heathcote, 1968).

Pettit and Taper (1968) examined the aflatoxins accumulated in spanish peanut kernel samples and found that out of 334 samples, 239 were negative, 73 had from a trace to 6 ppb, 13 had from 6 to 29 ppb, and 9 showed 30 ppb to a maximum of 3700 ppb. In general, peanuts harvested from cultivated lands with peanuts in the previous year were more highly infected with fungi and contained more aflatoxins than peanuts grown on land previously planted with rye, oats, melons, or potatoes. Mirocha (1982) noted that peanuts or groundnuts have received the greatest attention in terms of contamination by aflatoxins produced either by A. flavus or A. parasiticus. However, it is equally true that in some geographic areas of the world, e.g. southeast United States, corn is a high risk crop.

Tung and Ling (1968)collected peanut samples from various industrial sources in Taiwan and found that three of eight peanut samples, 9 of 13 peanut butter preparations, 4 of 12 sources of peanut cakes and 8 of 17 peanut oils contained aflatoxins. It should be noted that in Taiwan, peanut oil is produced without alkali refining, a procedure used in the United States which was effective in degrading aflatoxins. The same investigators tested three peanut butter preparations imported from the United States and found them aflatoxins free.

Aflatoxins in commercial feeds containing peanut meal have been implicated in mycotoxicoses involving a variety of animals : pigs (Lim, 1964 & Pettit & Taber 1968), mink (Astrakhantsev, 1967), nutria (Stefan, 1967), ducks and chickens (John & Uhlcmann, 1967).

The most extensive reported survey of cereal grains and soybeans seeds from commercial channels for the presence of aflatoxins was carried out by Shotwell et al., (1969a,b). Very low levels of wheat appeared to be contaminated with aflatoxin (2-19 ppb) and were detected by thin layer chromatography (TLC) in a total of nine out of 1368 samples of wheat grain, sorghum, and oat samples assayed. However, none of these positive samples were definitely confirmed by the duckling test. A total of 1311 corn samples and 866 soybean samples including samples from all grades and two different crop years assayed for toxins. The sensitivity limit was 2 - 5 ppb of the 35 corn samples. They were positive by TLC, 30 gave aflatoxins like responses in the duckling; five of these were in grade 5 and 25 in sample grade. Two of the 866 soybeans apparently did not constitute a good substrate for toxins production. Twenty-one samples of retail milk in South Africa were analyzed for aflatoxin M. Five were positive, two samples contained 160 mg/kg milk (Purchase & Vorster, 1968).

Saito et al., (1984) examined the natural occurence of aflatoxins and aflatoxicols in commercial pistachio nuts, corn, and corn flours. Aflatoxin B_1 and B_2 were detected in all 7 samples of pistachio nuts at 2.0-800 and 0.4-180 ppb, respectively. Aflatoxins G_1 and G_2 were detected in 4 samples at 0.6-51.4 and 0.2-16.3 ppb, respectively. Aflatoxin M_1 was detected in 5 samples at 1.8-39.3 ppb. Aflatoxicol I was detected in 5 samples at 3.2-26.3 ppb. Aflatoxins B_1 and B_2 were detected in all the 5 samples of corn flowers at 131-340, 5.3-9.9 ppb, and in 2 samples of feed corn at 131-340 and 17.1-46.9 ppb, respectively. Corn flowers and feed corn contained no aflatoxins G_1, G_2. Aflatoxicols I and II were deteced in 2 samples of feed corn at 13.5-25.0, 12.9-25.4, and 7.9-15.7 ppb. respectively. Corn flowers contained no aflatoxin M, or aflatoxicols I and II. Aflatoxicols were identified from their acetate derivs and by their fluorescence spectra.

Dutton and Wstlake (1985) studied the occurrence of mycotoxins in cereals and animals feedstuffs in Natal, South Africa using 800 samples of agricultural commodities. Aflatoxin B_1 showed the highest incidence rate; it occurred in $>27\%$ of all samples analyzed. The highest levels being found in peanut meal at 1500 ppb. Other mycotoxins detected were patulin and a number of trichothecene toxins at incidence rate in all commodities of 5.6 and 3.1% respectively. The commodities at highest risk were oil-seeds, excluding soyabean; the latter was found to be fairly free from contamination with mycotoxins. The most prevalent fungi isolated were Aspergillus flavus and A. parasiticus. They were found in $>22\%$ of all samples, wheras Penicillium showed the lowest incidence, and identified in 8.3% of all samples examined. This latter finding explains in part the low incidence of Penicillium mycotoxins.

Sabino et al., (1989) studied the levels of aflatoxins and zearalenone in 328 samples of corn by TLC. In 12.3% of these samples aflatoxin B_1 was detected in concentration that varied from 10 to 900 ug/kg (ppb). 18 samples showed levels above those tolerated by Brazilian legislation. Zearalenone was found in 4.5% of the samples analysed in concentrations that varied from 653 to 9830 ug/kg (ppb). The limit of detection of the method for the determination of zearalenone was 260 ug/kg (ppb) and the recovery was 100%.

A survey of the occurrence of aflatoxins B_1, B_2, G_1 and G_2 in soviet domestic and imported cereals and nuts (totalling 4532 samples) collected in 1985-1987 devised by Tutelyan et al., (1989) showed that

26.9% of imported peanuts, 2.2% of corn and 28.3% of cotton seeds were contaminated by aflatoxins at levels excedding the maximum tolerated level established in USSR. These were 5 ug/kg for aflatoxin B_1 in foodstuffs of all types excluding baby foods, maximum concentrations were 3650, 600 and 153 ug/kg respectively. A highly sensitive normalphase high-performance liquid chromatographic method was developed. The detection limit was 0.1 ug/kg and the coefficients of variation were 11% and 8.5% at contamination levels 10 and 100 ug/kg of aflatoxin B_1 respectively.

Sebunya and Rourtee (1990) showed that the populace was exposed to consumption of aflatoxins contaminated foods in Uganda. These studies also linked the highest incidence of liver cancer in the world to the presence of high levels of aflatoxins in the food and beverages. After a lapse of a decade, it was of interest to investigate the occurrence of aflatoxins and aflatoxigenic fungi in staple Ugandan food crops and poultry feeds derived from these foodstuffs. Fifty-four samples consisting of corn and peanuts, soybean and poultry feed were analyzed for the presence of aflatoxigenic A. flavus and A. parasiticus. 25 of the samples were also screened for aflatoxins B_1 and G_2, zearalenone, sterigmatocystin, ochratoxin A, citrinin, vomitoxin, and diacetoxyscirpenol (D.A.S.). Aflatoxigenic A. flavus/parasiticus was detected from the majority of corn (77%), peanuts (36% human food and 83.3% animal feed) and poultry feed (66.6%) but not from